consult. Studies were heterogeneous with regard to the predefined use of appropriate nephrology consultation indications; one study used discharge from nephrology care within one year of consultation as the definition of an inappropriate use of resources.[7] Two studies evaluated changes in the timing of referral relative to the onset of chronic kidney failure following reporting, but the use of significantly different definitions of early and late consultation complicated the assessment.[7]

Assessment of the future impact of eGFR reporting

These preliminary studies are encouraging in the overall trend for a benefit of eGFR reporting. Data are needed on the impact of reporting on patient and public awareness of CKD and its risk factors and outcomes.[10] Evaluation of the impact of reporting will be essential for the refinement of methods of estimating GFR, such as the CKD Epidemiology Collaboration (CKD-EPI) 2009 creatinine equation and the 2012 CKD-EPI cystatin C equation. It will also be important to investigate patient safety, but the metrics will need to be more precisely defined. What is the impact of eGFR reporting on the timing of dialysis initiation? Will there be an influence of reporting when enough time has elapsed to accrue adequate hard end points for cardiovascular events, onset of chronic kidney failure, and mortality? Future studies of eGFR reporting should further explore the impact of the prompt as a form of clinical decision support. There is advance notice to consider the implementation before publication of an updated CKD CPG anticipated in 2012 from Kidney Disease: Improving Global Outcomes and the corresponding K/DOQI US Commentary. This will provide an opportunity to reframe the discussion regarding the controversies and adoption challenges for routine primary and nephrology CKD care in a second cycle of development, publication, and implementation.

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REFERENCES

1. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**(Suppl 1): S1–S266.
2. De Coster C, McLaughlin K, Noseworthy TW. Criteria for referring patients with renal disease for nephrology consultation: a review of the literature. *J Nephrol* 2010; **23**: 399–407.
3. Kagoma YK, Garg AX, Li L *et al.* Reporting of the estimated glomerular filtration rate decreased creatinine clearance testing. *Kidney Int* 2012; **81**: 1245–1247.
4. Jain AK, McLeod I, Huo C *et al.* When laboratories report estimated glomerular filtration rates in addition to serum creatinines, nephrology consults increase. *Kidney Int* 2009; **76**: 318–323.
5. Hemmelgarn BR, Zhang J, Manns BJ *et al.* Nephrology visits and health care resource use before and after reporting estimated glomerular filtration rate. *JAMA* 2010; **303**: 1151–1158.
6. Wyatt C, Konduri V, Eng J *et al.* Reporting of estimated GFR in the primary care clinic. *Am J Kidney Dis* 2007; **49**: 634–641.
7. Kagoma YK, Weir MA, Iansavichus AV *et al.* Impact of estimated GFR reporting on patients, clinicians, and health-care systems: a systematic review. *Am J Kidney Dis* 2011; **57**: 592–560.
8. den Hartog JR, Reese PP, Cizman B *et al.* The costs and benefits of automatic estimated glomerular filtration rate reporting. *Clin J Am Soc Nephrol* 2009; **4**: 419–427.
9. Tawadrous D, Shariff SZ, Haynes RB *et al.* Use of clinical decision support systems for kidney-related drug prescribing. *Am J Kidney Dis* 2011; **57**: 592–560.
10. Stevens LA, Levey AS. Impact of reporting estimated glomerular filtration rate: it's not just about us. *Kidney Int* 2009; **76**: 245–247.

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Double-edged sword: a p53 regulator mediates both harmful and beneficial effects in experimental acute kidney injury

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Acute kidney injury triggers activation of innate immune responses and of proapoptotic programs such as the p53 pathway. Mulay *et al.* examine the effects of blocking murine double minute-2 (mdm2), a negative regulator of p53, using a novel chemotherapeutic agent, nutlin-3a, in mouse ischemia–reperfusion injury. Their results indicate that mdm2 promotes renal regeneration by limiting p53-mediated apoptosis but also enhances early inflammation by facilitating DNA binding of nuclear factor-κB independently of p53.

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There continues to be intense interest in furthering our understanding of the cellular and molecular mechanisms underlying acute kidney injury (AKI) through the use of animal models.[1] Although attempts at translation of laboratory research findings to patient populations with AKI have yet to yield dramatic clinical benefits, there is now a clearer appreciation of the complexity of this challenge.[1] There is also an emerging consensus that robust preventative or therapeutic interventions may require the manipulation of multiple pathways to renal injury—either simultaneously or at different stages of the process.[1] Two mechanisms of injury that may be of specific interest in this regard are the

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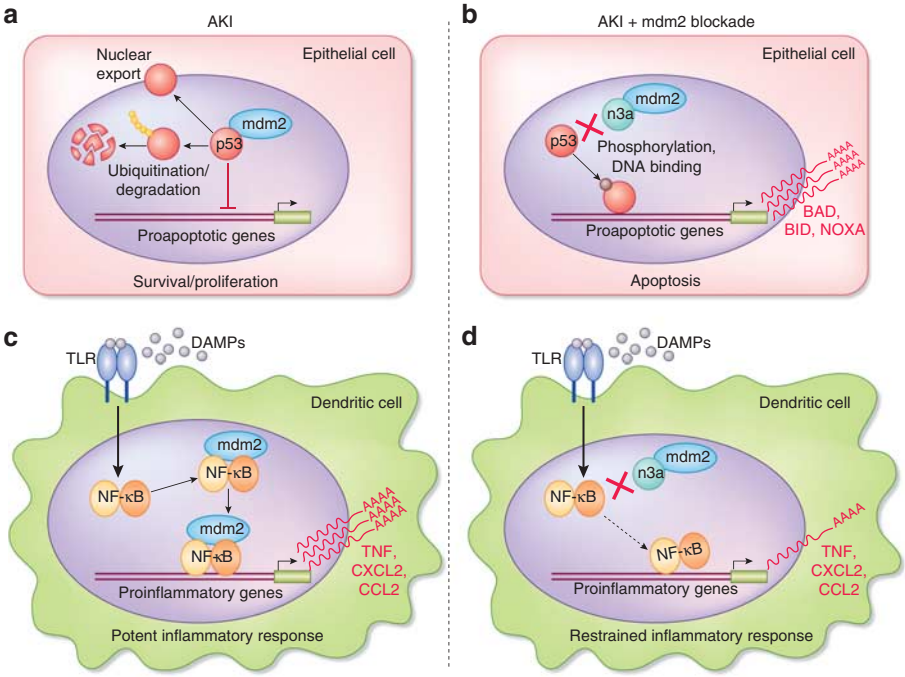

Figure 1 | Dual effects of murine double minute-2 blockade during acute kidney injury based on the results of Mulay *et al.*[4] (**a**) In its best-described role, murine double minute-2 (mdm2) interacts with p53 via an N-terminal domain and targets p53 for nuclear export or, by E3 ubiquitin ligase activity, for proteosomal degradation. During acute kidney injury (AKI), this function of mdm2 may limit p53-mediated upregulation of proapoptotic genes and, by so doing, promote tubular epithelial cell survival and proliferation during the regenerative phase. (**b**) In the presence of the mdm2 blocker nutlin-3a (n3a), p53-mediated transcription of proapoptotic gene products such as BAD, BID, and NOXA is enhanced, favoring increased epithelial cell apoptosis and inferior recovery of kidney function. (**c**) In cells of the innate immune system such as intrarenal dendritic cells, mdm2 may facilitate binding of the transcription nuclear factor-κB (NF-κB) to promoter regions of genes encoding proinflammatory mediators such as tumor necrosis factor (TNF), CXCL2, and CCL2 in response to Toll-like receptor (TLR) signaling initiated by release of danger-associated molecular patterns (DAMPs). This property of mdm2 promotes a potent inflammatory response that may exacerbate early tissue damage and cell loss during AKI. (**d**) For this mechanism of action of mdm2, the inhibitor nutlin-3a is associated with attenuated NF-κB-driven transcription of proinflammatory genes, resulting in a restrained inflammatory response and reduced early tissue injury and loss of kidney function.

intense immune/inflammatory response that is initiated early in the course of diverse forms of AKI and the activation of intracellular pathways associated with apoptotic cell death.[1,2] A corollary to this point is that the failure of some individual therapies to translate into improved AKI outcomes in human clinical trials may be due to opposing beneficial and detrimental effects on different components of the injury response. Finally, while it is clear that the various common causes of AKI trigger an overlapping set of tissue response programs,[1] some forms of renal damage, such as cancer chemotherapy-associated nephrotoxicity,[3] may be associated with discrete primary mechanisms that will allow tailored therapies or preventative measures to be developed.

Mulay *et al.*[4] (this issue) provide novel insights into AKI pathogenesis that strikingly underscore both the value of animal model experimentation in this field and the complexity of applying such insights to the clinical setting. The study, from the laboratory of Hans-Joachim Anders, examines the role of murine double minute-2 (mdm2)—an E3 ubiquitin ligase known to negatively regulate the proapoptotic transcription factor p53[5]—in mouse ischemia–reperfusion injury (IRI). An important rationale for the study was the recent development of mdm2 inhibitors such as nutlin-3a for clinical use as anticancer agents based on their ability to enhance p53-driven upregulation of proapoptotic gene products in neoplastic cells.[6] Given that expression of p53 is known to be increased in renal parenchymal cells during AKI and to have a detrimental impact on renal tubular regeneration,[7,8] the authors logically hypothesized that stabilization and activation of p53 by nutlin-3a would exacerbate AKI by exaggerating the apoptotic response in renal parenchymal cells (Figure 1a and b). In keeping with this, they observed that nutlin-3a further increased total and phosphorylated p53 levels after IRI with resultant increases in intrarenal transcript levels of p53-dependent proapoptotic proteins such as BAD, BID, and NOXA. Furthermore, blood urea nitrogen and tubular injury scores were increased and proximal and distal tubular cell viability was decreased during the 'regeneration phase' (5 days post-IRI) in nutlin-3a-treated animals—an effect that was absent in p53-deficient mice. These findings support the concern that patients receiving nutlin-3a therapy may experience heightened susceptibility to acute renal failure following nephrotoxic insults as a direct result of increased p53 activation. They are also consistent with the findings of Molitoris *et al.*, who performed *in vivo* siRNA knockdown of p53 in the kidney following IRI and cisplatin nephrotoxicity and observed dose-dependent improvements in post-AKI renal function, cortical and medullary tubular necrosis, and number of apoptotic cells.[8]

However, the p53-dependent disruption of tubular regeneration was not the only effect of mdm2 blockade observed by Mulay *et al.* following IRI.[4] Nutlin-3a administration was also associated with a striking reduction in early loss of renal function and with reduced intrarenal inflammation. In contrast to the day 5 results, at 24 h after renal artery clamping, animals receiving nutlin-3a had lower serum creatinine and blood urea nitrogen concentrations compared with vehicle-treated controls. At this same time point, their kidneys exhibited reduced tubular injury and apoptotic cell numbers, increased proximal and distal tubular epithelial cell viability, reduced neutrophil infiltration, and reduced mRNA levels of the proinflammatory mediators tumor necrosis factor (TNF), CXCL2, CCL2, and interleukin-6. Interestingly, the increased

intrarenal expression of mRNA for TNF, CXCL2, and CCL2 that occurred at 24 hours after IRI was localized by cell separation to $CD11c^+$ dendritic cells and was specifically diminished in these cells in nutlin-3a-treated animals. These unanticipated results imply that mdm2 functions in some fashion as a positive regulator of the proinflammatory response to tissue injury—perhaps specifically in tissue resident mononuclear phagocytes such as renal dendritic cells (Figure 1c and d). They also indicate a potential for the use of mdm2 inhibitors in immune/inflammatory diseases—a possibility that is strengthened by the same group's recent report of beneficial effects of nutlin-3a on autoimmune tissue injury in the MRL-Fas^{lpr} model of systemic lupus erythematosis.[9]

The observed anti-inflammatory effects of nutlin-3a in both AKI and lupus models evoke important questions about the mechanism whereby mdm2 enhances immune/inflammatory responses and the potential for this mechanism to be separated, for therapeutic purposes, from its role in preventing p53-mediated apoptosis. It is worth, therefore, considering the molecular interactions and functions of mdm2 in more detail. In its best-characterized role, mdm2 inhibits transactivation of p53, exports p53 out of the nucleus, and promotes proteosomal degradation of p53 through its E3 ubiquitin ligase activity (Figure 1a).[5,6] The p53–mdm2 interaction occurs between the N-terminal domains of both proteins. This interaction is disrupted by nutlin-3a, leading to accumulation and activation of p53, which controls the cellular response to endogenous injury by upregulating expression of cell-cycle, apoptotic, DNA repair and senescence genes that determine cell fate (Figure 1b).[6] In addition, however, mdm2 has been reported to regulate the expression and/or degradation of a range of target proteins independently of its interaction with p53. Such targets include the retinoblastoma susceptibility gene product pRB, the transcription factors E2F1 and Foxo3A, the antiapoptotic protein XIAP, and E-cadherin.[5] Some of these interactions clearly involve domains of the mdm2 protein that are distinct from the p53-binding N-terminus.[5] It has also been noted, however, that high doses of nutlins affect proliferation of tumor cells that lack p53, indicating that modulation of mdm2 at its p53-binding site affects its activity on targets independent of p53.[6]

To begin to elucidate the mechanism of reduced early inflammation in their IRI experiments, Mulay *et al.* have conducted a careful series of experiments, focusing primarily on nuclear factor-κB (NF-κB)-mediated upregulation of proinflammatory mediators.[4] Lipopolysaccharide (LPS) stimulation of p53-deficient and p53/mdm2-double-deficient mouse embryonic fibroblasts was used to determine whether p53 was necessary for mdm2-dependent induction of interleukin-6 and TNF. Interestingly, while LPS-induced secretion of IL-6 and TNF occurred robustly in p53-deficient cells, combined deficiency of p53 and mdm2 resulted in the virtual absence of cytokine production. Despite this striking difference, proximal signaling events associated with LPS stimulation were intact in the double-deficient cells, including phosphorylation and degradation of IκB, nuclear translocation of the NF-κB proteins p65 and p52, and activation of mitogen-activated protein kinase signaling pathways. In contrast, it was found that LPS-induced recruitment of NF-κB to the *il-6* gene promoter and transcriptional activity from an *il-6* promoter-driven reporter construct were reduced in the combined absence of p53 and mdm2. On this basis, the authors conclude that the proinflammatory function of mdm2 inhibition is (1) independent of p53 and (2) the result of an intranuclear mechanism whereby mdm2 facilitates binding of NF-κB dimers to promoter regions of key target genes (Figure 1c). Whether this mechanism of action reflects direct interaction between mdm2 and NF-κB proteins in the nucleus or is mediated indirectly by an effect of mdm2 on the accessibility of promoter DNA via, for instance, histone-modifying enzyme systems[10] will require further study. It should also be noted that additional mechanisms for mdm2-mediated enhancement of NF-κB-driven inflammatory gene transcription have been reported. For example, in a model of LPS-induced acute lung injury, treatment of mice with nutlin-3a resulted in reduced severity of lung injury and its associated inflammatory components. In the same study, culture of neutrophils and macrophages with nutlin-3a decreased LPS-induced NF-κB-binding activity, but this effect was dependent on the presence of p53.[11] Given the complexity of the molecular interactions of both mdm2 and p53 as well as the diversity of their potential effects on protein stability, intracellular signaling events, and gene transcription,[5,6] it is possible that the anti-inflammatory effects of mdm2 inhibition involve multiple mechanisms that differ by cell type and context.

The results reported by Mulay *et al.* in this comprehensive study[4] are compelling for both the clinical nephrologist and the renal physiologist. Although the potential detrimental effect of the novel chemotherapeutic agent nutlin-3a on renal regeneration following AKI is demonstrated, we have also learned that mdm2 inhibition holds real promise as a strategy to reduce tissue damage associated with renal inflammation.[4,9] The findings provide a strong impetus for additional biochemical and *in vivo* experiments aimed at more precisely defining the molecular details of mdm2-mediated enhancement of proinflammatory mediator expression, particularly in immunological cells such as renal dendritic cells. The clinician may also note that the law of unintended consequences, so frequently encountered during the management of patients with renal failure, is well illustrated here: what may appear to be a useful outcome in the early stages of an illness (decreased immune system activation) can prove to be disappointing in the long term (impaired regeneration as a result of increased cellular senescence and apoptosis). The study of Mulay *et al.*[4] does, however, provide reason to believe that continued progress in understanding the molecular pathways linking immune/inflammatory response with epithelial cell apoptosis and regeneration throughout the course of AKI will serve as a basis for more successful therapeutic strategies in the future.

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REFERENCES

1. Kinsey GR, Okusa MD. Pathogenesis of acute kidney injury: foundation for clinical practice. *Am J Kidney Dis* 2011; **58**: 291–301.
2. Havasi A, Borkan SC. Apoptosis and acute kidney injury. *Kidney Int* 2011; **80**: 29–40.
3. Sahni V, Choudhury D, Ahmed Z. Chemotherapy-associated renal dysfunction. *Nat Rev Nephrol* 2009; **5**: 450–462.
4. Mulay SR, Thomasova D, Ryu M *et al.* MDM2 (murine double minute-2) links inflammation and tubular cell healing during acute kidney injury in mice. *Kidney Int* 2012; **81**: 1199–1211
5. Manfredi JJ. The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. *Genes Dev* 2010; **24**: 1580–1589.
6. Shangary S, Wang S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel approach for cancer therapy. *Annu Rev Pharmacol Toxicol* 2009; **49**: 223–241.
7. Kelly KJ, Plotkin Z, Vulgamott SL *et al.* P53 mediates the apoptotic response to GTP depletion after renal ischemia-reperfusion: protective role of a p53 inhibitor. *J Am Soc Nephrol* 2003; **14**: 128–138.
8. Molitoris BA, Dagher PC, Sandoval RM *et al.* siRNA targeted to p53 attenuates ischemic and cisplatin-induced acute kidney injury. *J Am Soc Nephrol* 2009; **20**: 1754–1764.
9. Allam R, Sayyed SG, Kulkarni OP *et al.* Mdm2 promotes systemic lupus erythematosus and lupus nephritis. *J Am Soc Nephrol* 2011; **22**: 2016–2027.
10. Zager RA, Johnson ACM. Renal ischemia-reperfusion injury upregulates histone-modifying enzyme systems and alters histone expression at proinflammatory/profibrotic genes. *Am J Physiol Renal Physiol* 2009; **296**: F1032–F1041.
11. Liu G, Park Y-J, Tsuruta Y *et al.* p53 attenuates lipopolysaccharide-induced NF-κB activation and acute lung injury. *J Immunol* 2009; **182**: 5063–5071.

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Does IgA antibody against β2 glycoprotein I increase cardiovascular risk in hemodialysis patients?

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Cardiovascular disease is the most common cause of mortality in patients with chronic kidney disease on hemodialysis. In addition to a high prevalence of traditional cardiovascular risk factors, other specific factors, including uremia and chronic inflammation, seem to contribute to the excess cardiovascular mortality. The findings of Serrano *et al.* point to a link between IgA antibodies against β2 glycoprotein I and cardiovascular events in renal dialysis patients.

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The risk of cardiovascular disease is high in patients with chronic kidney disease, and cardiovascular disease accounts for up to 50% of deaths in this population. In the subset of patients receiving hemodialysis the rate of cardiovascular mortality is 10–to 20–fold higher than that of the general population.[1] In addition to a high prevalence of traditional cardiovascular risk factors in patients with chronic renal disease (for example, hypertension and diabetes mellitus), other specific risk factors, including degree of uremia, comorbidity, inflammatory response, hypoalbuminemia, and hyperparathyroidism, also seem to contribute to the excess cardiovascular risk.

The antiphospholipid syndrome (APS) is an acquired, strongly prothrombotic disease characterized by arterial and venous thromboembolism and/or pregnancy morbidity in the presence of persistent high-titer autoantibodies directed against a broad range of phospholipids and phospholipid-binding proteins.[2] The history of APS dates back to reports in the 1950 s of false-positive laboratory test results for syphilis in patients who went on to develop systemic lupus erythematosus (SLE). It became apparent that Wasserman *et al.* had detected the earliest anti-phospholipid antibodies in 1906 with the development of a complement-fixation assay for syphilis using phospholipid antigen from hepatic extract of fetuses with congenital syphilis.[3] This phospholipid antigen was later named 'cardiolipin' (as subsequently mitochondrial phospholipids were extracted from bovine heart muscle), and anti-cardiolipin antibody detection became the basis of the current-day Venereal Disease Research Laboratory (VDRL) test for syphilis. Widespread screening of donated blood for syphilis led to the realization that some patients with SLE possessed anti-phospholipid antibodies resulting in false-positive VDRL tests, prolonged laboratory clotting times, and strong predisposition to thrombosis.

Anti-phospholipid antibodies are detected either by a prolongation of phospholipid-dependent coagulation tests (lupus anticoagulant) or by solid-phase immune assays. They are a heterogeneous group of autoantibodies directed against a variety of different antigens (including prothrombin, annexin V, protein C, and protein S); it is thought that binding of these antigens is at least partly responsible for the prothrombotic phenotype of APS. In the early 1990s, several groups reported that a proportion of anti-cardiolipin antibodies were directed against the phospholipid cofactor β2 glycoprotein I (β2GPI). This single-chain polypeptide glycoprotein is a 50–kDa plasma apolipoprotein (plasma concentration 200 μg/ml) that binds anionic phospholipids; it is required for anti-phospholipid antibody binding in a subset of patients with SLE

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